

AMENDMENTS TO THE SPECIFICATION

Replace paragraph 1 at page 1 with the following amended paragraph.

(0001) This application is a ~~divisional application~~ continuation application under
5 37 C.F.R. 1.53(b) and claims priority of pending U.S. application Serial No.
09/535,675, filed March 23, 2000, which ~~is a continuation-in-part of~~ claims priority
from abandoned U.S. provisional application Ser. No. 60/190,140, filed March 16,
2000, abandoned U.S. provisional application Ser. No. 60/164,048, filed
November 8, 1999, abandoned U.S. application Ser. No. 09/414,905, filed
10 October 8, 1999, abandoned U.S. provisional application Ser. No. 60/140,028,
filed June 16, 1999, and abandoned U.S. provisional application Ser. No.
60/126,056, filed March 23, 1999, all of which are incorporated herein by
reference.

15 Replace paragraph 35 at page 10 with the following amended paragraph.

(00035) Other embodiments include a method to enhance the expression of
one or more cytokines or interleukins that facilitate Th1 immune responses in a
subject or to reduce the expression of one or more cytokines or interleukins that
20 facilitate Th2 immune response in a subject comprising administering to the
subject an effective amount of the composition of claim 32, whereby the subject's
Th1 ~~immune response is enhanced or the~~ immune response is enhanced or the
subject's undesired Th2 immune response is reduced.

25 Replace paragraph 135 at page 40 with the following amended paragraph.

(000135) Another related embodiment is BrEA hemihydrate that is milled to
an average particle size of about 0.01-200 μ M, or about 0.1-10 μ M or about 0.5-5
 μ M. Average particle size or diameter for milled BrEA hemihydrate may thus be
30 relatively small, e.g., about 0.03-2.0 μ M or about 0.1-1.0 μ M, or ~~somewhat larger,~~
~~e.g., about about~~ somewhat larger, e.g., about 0.5-5.0 μ M or about 1-5.0 μ M.

Milled BrEA hemihydrate is suitable for preparing solid formulations and parenteral formulations for human or veterinary use. The milled material facilitates dissolution of BrEA hemihydrate in solvents or excipients and facilitates mixing with solids or solid excipients.

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Replace paragraph 202 at page 79 with the following amended paragraph.

(000202) **Groups 37-1 through 37-25-10-6.** These groups comprise each compound named in all of the ~~compound-compound groups~~ the compound
10 groups 1 through 36-25-10-6 described above wherein R¹ is not divalent, e.g., is not =O, and it is in the α -configuration, instead of the β -configuration as shown in formula B.

15 Replace paragraph 206 at page 80 with the following amended paragraph.

(000206) **Groups 41-1 through 41-25-10-6.** These groups comprise each compound named in all of the compound groups 1 through 36-25-10-6 described above ~~wherein R² and R⁴ is not divalent, e.g., they is not~~ wherein R² and R⁴ are
20 not divalent, e.g., they are not =O, and they are both in the α -configuration, instead of the β -configuration as shown in formula B.

Replace paragraph 216 at page 238 with the following amended paragraph.

25 (000216) The formula A compounds, particularly where both R₁ at the 11-position are not hydroxyl, ~~alkoxy or a moiety~~ alkoxy or a moiety that can hydrolyze to a hydroxyl, are generally suitable for use in the methods and compositions that are disclosed herein, e.g., their use to enhance a subject's Th1 immune responses. Methods of administration and dosages are
30 essentially as described herein.

Replace paragraph 238 at page 101 with the following amended paragraph.

5 **(000238)** Treatment of 10a with LDA, followed by alkylation of the enolate allows introduction of side chains such as R^{10} , ~~which may be, e.g.,~~ which may be, e.g., C1-C20 alkyl (methyl, ethyl), C1-C20 alkenyl ($CH_2=CH-(CH_2)_{0-6}$), benzyl, $-(CH_2)_{1-4}-O-(CH_2)_{0-4}-CH_3$.

10 Replace paragraph 241 at page 103 with the following amended paragraph.

15 **(000241)** Phosphothioesters, $R^B O-P(SR^{PR})(O)-O-$ are generated by treatment of alcohols with the monothio analog of diethylchlorophosphate as described for phosphoesters yielding the phosphothioesters. Carbonates, $R^B O-C(O)-O-$ are generated from the corresponding steroid alcohol using the chloroformate ($R^B-C(O)-Cl$), e.g., C₁₋₂₀ alkyl, alkenyl or alkynyl chloroformates (e.g. $CH_3(CH_2)_{0-5}-C(O)Cl$). Carbamates, $R^B-NH-C(O)-O-$ are made from steroid alcohols by treatment with isocyanates ($R^B N=C=O$) or NaOCN in the presence of ~~trifluoroacetic acid. Amino acid esters~~ trifluoroacetic acid. Amino acid esters,
20 $ZNX-CHY-C(O)-O-$ are generated by coupling the steroid alcohol with the acid chloride of the N-protected amino acid.

25 Replace paragraph 245 at page 104 with the following amended paragraph.

30 **(000245)** Amines and derivatives of amines, e.g., $R^B NH-$, $R^B-C(O)NH-$, $R^B OC(O)-NH-$ or $R^B O-C(O)-CH R^B-NH-$ linked to steroid carbon atoms, are typically prepared by standard methods. For example, amines (NH_2 -steroid) are generally prepared using the Hoffmann rearrangement (Br_2 , NaOH) from the amide ($NH_2-C(O)$ -steroid) or the Curtius rearrangement (NaN_3) from the acid chloride of the steroid. The R^B substituent can subsequently be introduced by

alkylation. Steroid alcohols can be used as starting materials under standard Mitsunobu conditions (PPh_3 , DEAD) to yield N-Boc sulfonamides using N-(t-butoxycarbonyl)-p-toluenesulfonamide. One can selectively remove either protecting group. Treatment with trifluoroacetic acid affords the sulfonamide (R^{B} -S(O)(O)-NH-steroid). Alternatively, sodium naphthalenide deprotects to give the N-Boc compound. Amines (NH_2 -steroid) can be converted to amides ($\text{R}^{\text{B}}\text{NH}-\text{C}(\text{O})$ -steroid) using acyl chlorides ($\text{R}^{\text{B}}-\text{C}(\text{O})-\text{Cl}$). Treatment with ethyl chloroformate gives the N-carbamate ($\text{R}^{\text{B}}\text{O}-\text{C}(\text{O})-\text{NH}$ -steroid). The amine (NH_2 -steroid) can be alkylated with an α -bromoester ($\text{R}^{\text{B}}-\text{C}(\text{O})-\text{CH}_2-\text{NH}_2$) to yield the amino acid substituted steroid yield the amino acid substituted steroid ($\text{R}^{\text{B}}-\text{O}-\text{C}(\text{O})-\text{CH}_2-\text{NH}$ -steroid).

Replace paragraph 249 at page 105 with the following amended paragraph.

15 (000245) Scheme 11. Formula 1 compounds that comprise two or three ring heteroatoms are prepared as shown in the schemes. In the scheme, X is $-\text{CH}_2-$, $-\text{NH}-$, $-\text{O}-$, or $-\text{S}-$; R^{40} is $-\text{H}$ or $-\text{Br}$; R^{41} is an organic moiety having about 12 carbon atoms or less, typically C1 – C8 optionally substituted alkyl (e.g., methyl, hydroxymethyl, ethyl, propyl) or C2 – C8 optionally substituted alkenyl having a single double bond (e.g., vinyl) with 1, 2, 3 or ~~more independently selected~~ substituents more independently selected substituents (e.g., $-\text{OH}$, $-\text{COOH}$, $-\text{O}-$) and with any substituents that comprise a functional group generally being protected. Preparation of compound 20 from 19 is accomplished using a glycol such as $\text{HOC}(\text{CH}_3)_2\text{C}(\text{CH}_3)_2\text{OH}$ in acid (H^+) (B.H. Lipshutz et al., *Synth. Commun.* 12: 267, 1982). The use of a bulky protecting group facilitates generation of a double bond at the 5-6 position over the 4-5 position.

30 Replace paragraph 263 at page 134 with the following amended paragraph.

(000263) More typically hydroxy protecting ~~groups include substituted groups~~ include substituted methyl ethers, substituted benzyl ethers, silyl ethers, and esters including sulfonic acid esters, still more typically, trialkylsilyl ethers, tosylates and acetates.

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Replace paragraph 315 at page 151 with the following amended paragraph.

(000315) Many cancers or malignancies are associated with an unwanted
10 Th2 immune response or a deficient Th1 response. An insufficient Th1 immune response may play a role in the capacity of malignant cells to escape immune surveillance. These conditions include non-small cell lung cancer, bronchogenic carcinoma, renal cell cancer or carcinoma, lymphoma, glioma, melanoma, pancreatic or gastric adenocarcinoma, ~~human papillomavirus associated~~ human
15 papillomavirus associated cervical intraepithelial neoplasia, cervical carcinoma, hepatoma and cutaneous T-cell lymphoma (mycosis fungoides, Sezary syndrome).

Replace paragraph 318 at page 153 with the following amended
20 paragraph.

(000318) Insufficient Th1 ~~immune responses are often~~ immune responses are
often associated with viral infection. Viral infections may arise from DNA or RNA viruses, e.g., herpesviruses, hepadnaviruses, adenoviruses, retroviruses,
25 togaviruses, alphaviruses, arboviruses, flaviviruses, rhinoviruses, papillomaviruses and/or pestiviruses. Exemplary viruses have been described. See, for example B. N. Fields, et al., editors, *Fundamental Virology*, 3rd edition, 1996, Lippencott-Raven Publishers, see chapter 2 at pages 23-57, including table 4 at pages 26-27, table 5 at pages 28-29, chapter 17 at pages 523-539,
30 chapters 26-27 at pages 763-916, chapter 32 at pages 1043-1108 and chapter 35 at pages 1199-1233. As used herein, retroviruses include human and animal

viruses, e.g., HIV-1, HIV-2, LAV, human T-cell leukemia virus I ("HTLV I"), HTLV II, HTLV III, SIV, SHIV, FIV, FeLV. Additional viruses, including their genogroups, clades, isolates, strains and so forth, that may establish a virus infection include human hepatitis C virus ("HCV"), human hepatitis B virus ("HBV"), human
5 hepatitis A virus ("HAV"), duck hepatitis virus, woodchuck hepatitis virus, human ("HPV", e.g., HPV 6, HPV 11, HPV 16, HPV 18, HPV 31, HPV 45) or animal papilloma viruses, Poliovirus, Herpes simplex virus 1 ("HSV-1"), Herpes simplex virus 2 ("HSV-2"), human Herpesvirus 6 ("HHV-6"), human Herpesvirus 8 ("HHV-8"), Dengue virus (types 1-4), Western Equine Encephalitis Virus, Japanese
10 Encephalitis Virus, Yellow Fever Virus and Bovine Viral Diarrhea Virus.

Replace paragraph 340 at page 163 with the following amended paragraph.

15 **(000340)** The formula 1 compounds typically interact with one or more biological ~~ligands to effect a biological response~~ ligands to effect a biological response. To facilitate the identification of candidate binding partners for the formula 1 compounds, one can use a radiolabeled formula 1 compound that is linked to a support, usually a solid support, as a means to recover the candidate
20 binding partners. The formula 1 compound can be linked to the support through, e.g., the 3-, 7-, 16- or 17-position of the steroid nucleus. Linking agents are known for such uses and include homobifunctional and heterobifunctional agents, many of which are commercially available. The linker one uses will typically comprise about 2-20 linked atoms. The linked atoms usually comprise
25 mostly carbon, with one, two or three oxygen, sulfur or nitrogen atoms that replace one or more carbon atoms. One can use a cDNA expression library that one has made from suitable cells or tissues as a source of candidate binding partners. The cells or tissues can be obtained from a mammalian or a vertebrate host, e.g., human, mouse, bird, primate, or from other sources, e.g., insects (e.g.,
30 *Drosophila*), other invertebrates (e.g., yeast, bacteria, *Mycoplasma sp.*, *Plasmodium sp.*, *Tetrahymena sp.*, *C. elegans*) or other organism groups or

species listed herein or in the cited references. Suitable tissues include skin, liver tissue or cells, including hepatocytes and Kupfer cells, fibrocytes, monocytes, dendritic cells, kidney cells and tissues, brain or other central nervous system cells or tissues, including neurons, astrocytes and glial cells, peripheral nervous system tissues, lung, intestine, placenta, breast, ovary, testes, muscle, including heart or myocyte tissue or cells, white blood cells, including T cells, B cells, bone marrow cells and tissues, lymph tissues or fluids and chondrocytes.

10 Replace paragraph 601 at page 208 with the following amended paragraph.

(000601) 57C. The method of embodiment 56C wherein the one or more second therapeutic agents is a protease inhibitor, a reverse transcriptase inhibitor, a viral, bacterial or parasite DNA or RNA polymerase inhibitor, an
15 antibacterial antibiotic or an antifungal agent, such as AZT, ddI, ddC, D4T, 3TC, a viral (e.g., HIV) fusion inhibitor, hydroxyurea, nelfinavir, saquinavir, ritonavir, indinavir, chloroquine, a chloroquine analog, amphotericin B, fluconazole, clotrimazole, isoniazid, dapson, rifampin, cycloserine, erythromycin, a tetracycline antibiotic, vancomycin, ethambutol, pyrazinamide, a fluroquinolone
20 (e.g., ciprofloxacin, norfloxacin), a cephalosporin antibiotic, a β -lactam antibiotic ~~or an aminoglycoside antibiotic~~ or an aminoglycoside antibiotic (e.g., streptomycin, kanamycin, tobramycin).

25 Replace paragraph 694 at page 238 with the following amended paragraph.

(000697) In the diagrams tables shown below baseline data is indicated by "BL" or by "pre".

Increased immunophenotypes after BrEA therapy

5	Phenotype	Baseline ^a	Course 1	Course 2	Course 3
	CD8+CD69+CD25-	18	54	56	75
	n=	(13)	(13)	(9)	(4)
	p=		<0.001	<0.001	0.04
10	CD8+CD16+CD38+	8	27	28	25
	n=	(10)	(10)	(4)	(4)
	p=		<0.001	0.047	0.02
15	CD8-CD16+	53	253	288	249
	n=	(12)	(12)	(4)	(2)
	p=		<0.001	0.02	0.04
20	Lin- HLA-DR+ CD11c+/CD123+	3.2	17.7	11.4 ^c	14.7 ^c
	n=	(10)	(10)	(5)	(4)
	p=		<0.001	0.02	0.04
25	IL2+CD4 ^d	3.14 ^e	29.25	31.42	13.59
	n=	13	13	3	4
	p=		<0.001	0.09	0.04
30	IL10+CD4 ^d	66	20.9	8.9	15.3
	n=	13	13	5	3
	p=		0.005	0.005	0.03
	Th1 Response ^d	17	66	64	53
	n=	(13)	(13)	(5)	(5)
	p=		0.001	0.033	0.025

35 ^aMedian values of cells/ μ L

^bpaired value t test

^cTest not available at baseline for patients receiving second and third courses, baseline value from initiation of 2nd course = 6.4

^d% of CD4

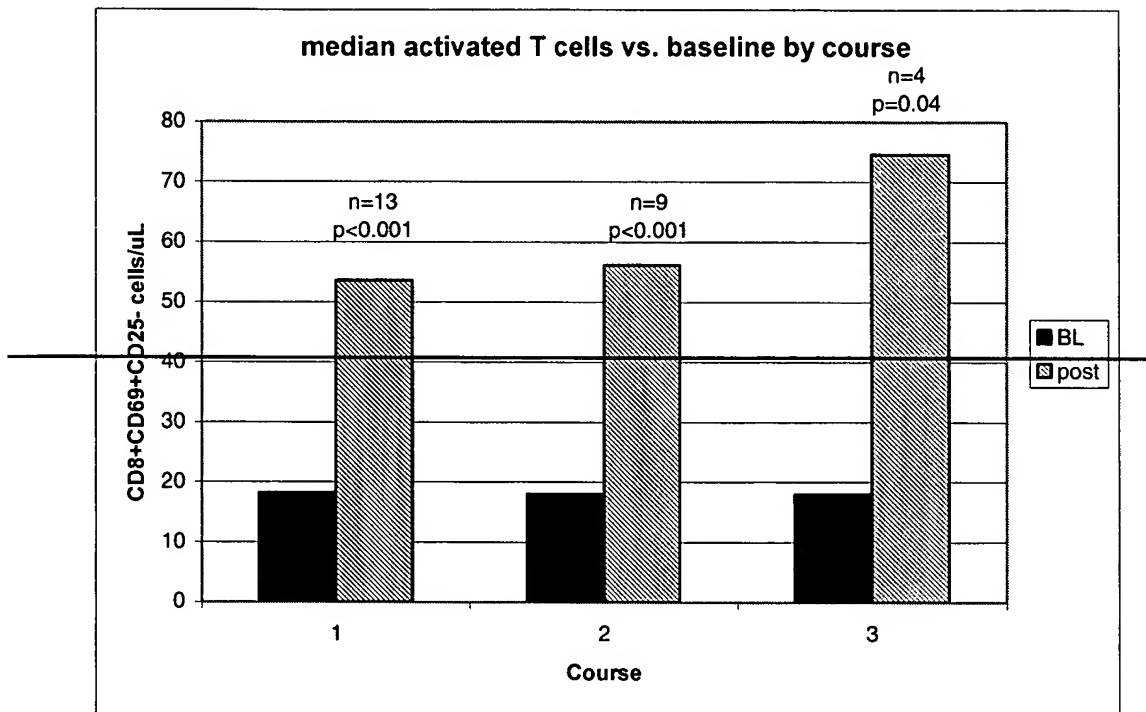
40 ^eBaseline values from day 8 (preceding the first five-day treatment)

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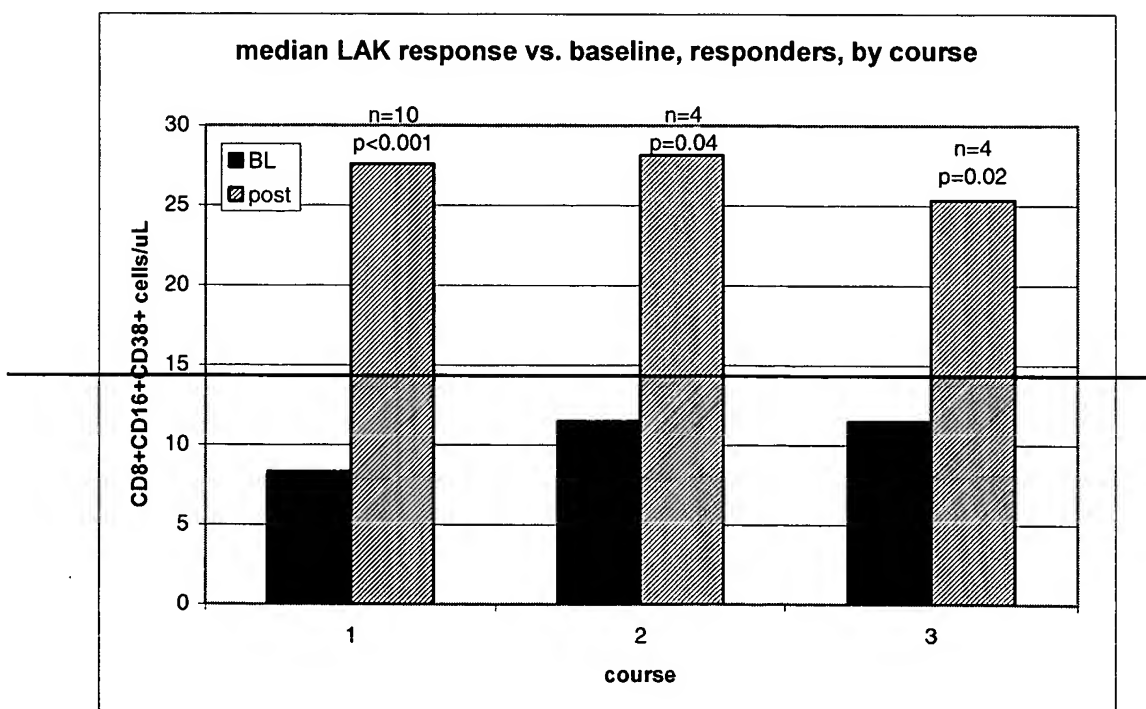
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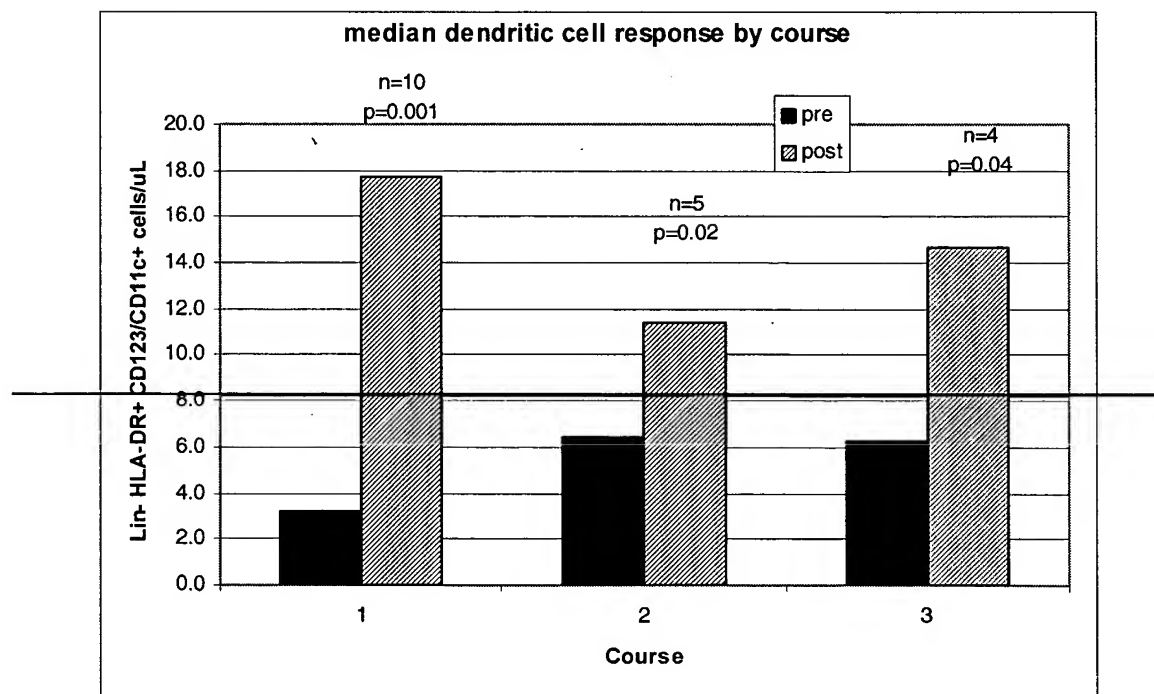
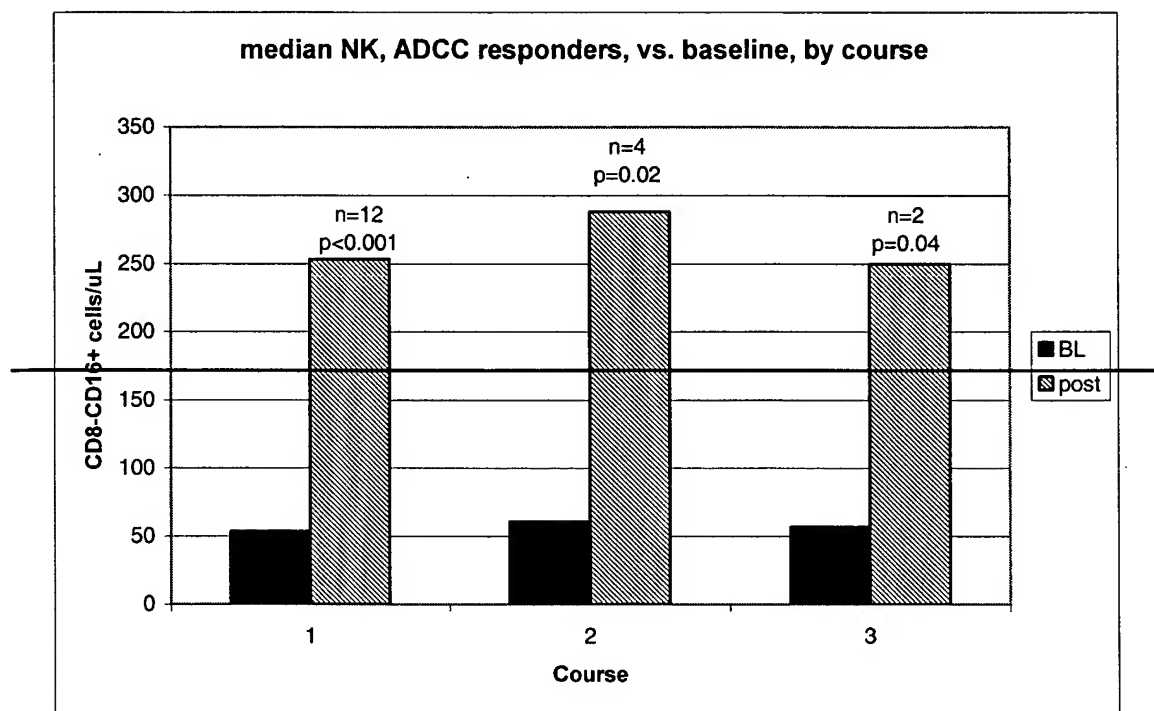
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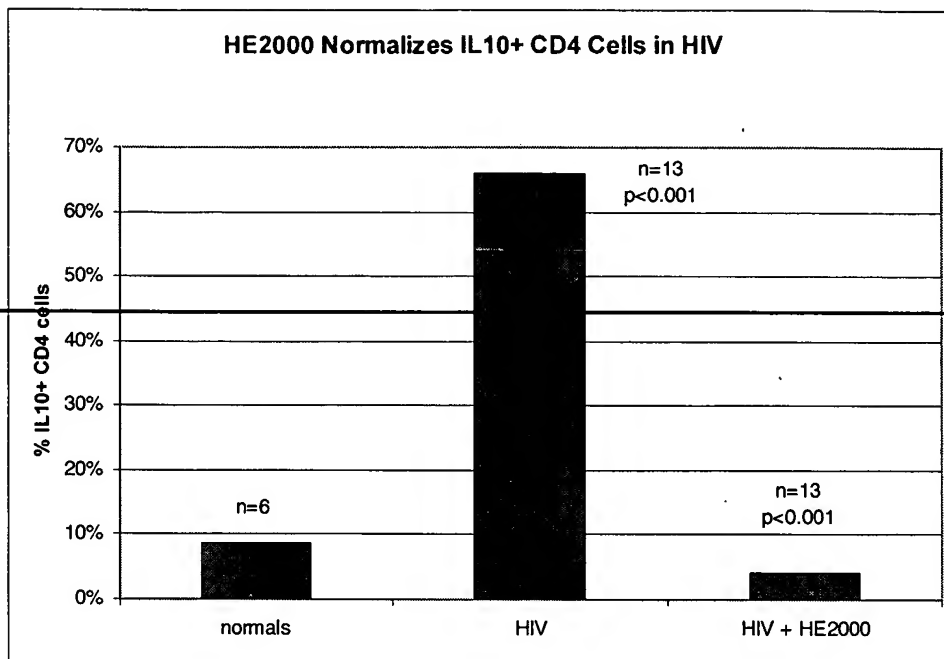




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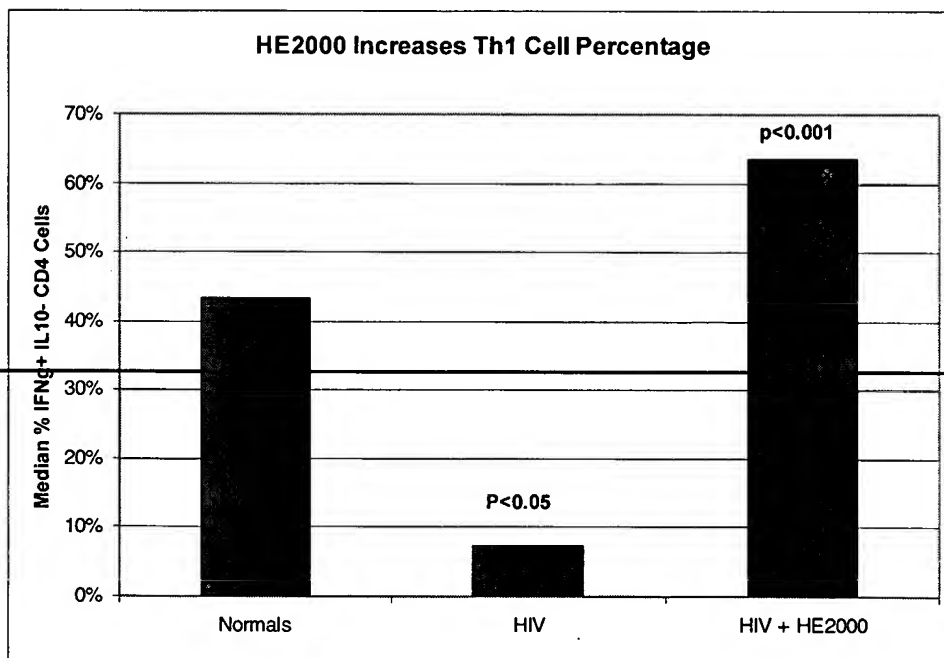
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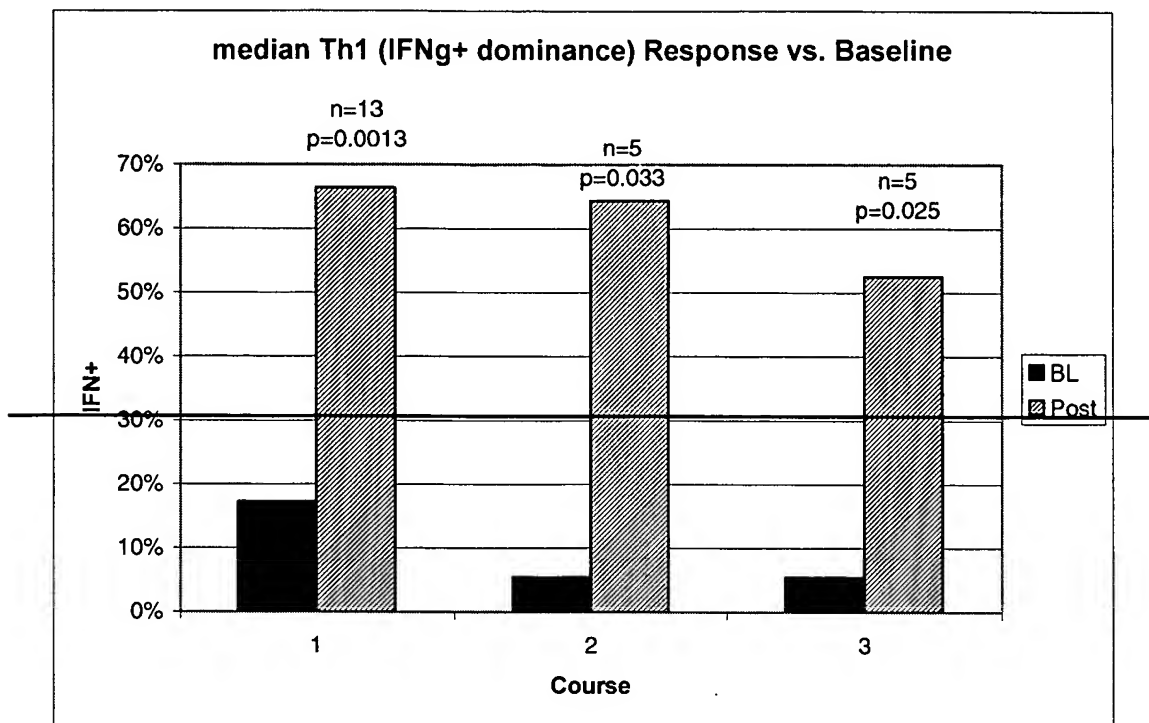
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Median activated T cells (CD8⁺CD69⁺CD25⁺) vs. baseline by course

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Course 1 baseline	19 cells/ μ L
Course 1	53 cells/ μ L n = 13, p < 0.001
Course 2 baseline	19 cells/ μ L
Course 2	54 cells/ μ L n = 9, p < 0.001
Course 3 baseline	19 cells/ μ L
Course 3	74 cells/ μ L n = 4, p = 0.04

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Median LAK response (CD8⁺CD16⁺CD38⁺) vs. baseline, responders, by course

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Course 1 baseline	8 cells/ μ L
Course 1	26 cells/ μ L n = 10, p < 0.001
Course 2 baseline	12 cells/ μ L
Course 2	27 cells/ μ L n = 4, p = 0.04
Course 3 baseline	12 cells/ μ L
Course 3	25 cells/ μ L n = 4, p = 0.02

Median NK, ADCC (CD8⁺CD16⁺) responders vs. baseline by course

	Course 1 baseline	52 cells/ μ L	
	Course 1	255 cells/ μ L	n = 12, p < 0.001
	Course 2 baseline	59 cells/ μ L	
5	Course 2	291 cells/ μ L	n = 4, p = 0.02
	Course 3 baseline	56 cells/ μ L	
	Course 3	249 cells/ μ L	n = 2, p = 0.04

Median dendritic cell response (Lin⁻HLA-DR⁺CD123⁺/CD11c⁺) by course

10	Course 1 baseline	3.2 cells/ μ L	
	Course 1	17.7 cells/ μ L	n = 10, p = 0.001
	Course 2 baseline	6.6 cells/ μ L	
	Course 2	11.6 cells/ μ L	n = 5, p = 0.02
	Course 3 baseline	6.3 cells/ μ L	
15	Course 3	14.7 cells/ μ L	n = 4, p = 0.04

16 α -Bromoepiandrosterone normalizes IL-10⁺ cells in HIV-infected patients

		% of CD4 ⁺ cells that are IL10 ⁺	
20	Normals (HIV ⁻)	8%	n = 6
	HIV ⁺ patients	64%	n = 13, p < 0.001
	treated HIV ⁺ patients	4%	n = 13, p < 0.001

25 16 α -Bromoepiandrosterone increases Th1 cell proportion in HIV-infected patients

		median % of CD4 ⁺ cells that are IFN γ ⁺ and IL10 ⁻	
	Normals (HIV ⁻)	43%	
	HIV ⁺ patients	8%	p < 0.05
	treated HIV ⁺ patients	63%	p < 0.001

Median Th1 (IFN γ ⁺ dominance) response vs. baseline

		median % of IFN γ ⁺ T cells	
	Course 1 baseline	17%	
35	Course 1	66%	n = 13, p = 0.00
	Course 2 baseline	5%	
	Course 2	64%	n = 4, p = 0.0
	Course 3 baseline	5%	
	Course 3	53%	n = 4, p = 0.0